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Tinkering with meiosis

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Abstract

Meiosis is at the heart of Mendelian heredity. Recently, much progress has been made in the understanding of this process, in various organisms. In the last 15 years, the functional characterization of numerous genes involved in meiosis has dramatically deepened our knowledge of key events, including recombination, the cell cycle, and chromosome distribution. Through a constantly advancing tool set and knowledge base, a number of advances have been made that will allow manipulation of meiosis from a plant breeding perspective. This review focuses on the aspects of meiosis that can be tinkered with to create and propagate new varieties.

We would like to dedicate this review to the memory of Simon W. Chan (1974-2012) (http://www.plb.ucdavis.edu/labs/srchan/).

Key words: apomixis, crossover, diploid gametes, haploid, meiosis, plant breeding, recombination, reverse breeding.

Introduction

In the coming decades, high-quality food must be produced to feed billions of people on this planet (Tester and Langridge, 2010). Research is making advances on how this may be possible by introgressing certain traits, more specifically alleles of genes, into the elite crop varieties that already exist. Methods are required to transfer genetic material between plant varieties. For this to occur, with the exception of transgenesis, the genetic material must pass by a peculiar cell division known as meiosis. Meiosis generates genetic diversity and maintains ploidy (the number of sets of chromosomes in a cell) through successive generations. During meiosis, chromosomes that will be transmitted to the next generation are recombined, redistributed, and their number then halved. Molecular data on plant meiosis has been accumulating rapidly in the last decade, mostly in the model species Arabidopsis thaliana, but notably also in rice (Oryza sativa) and maize (Zea mays). This has led to the functional characterization of over 60 genes involved in various aspects of meiosis, from recombination to the cell cycle and chromosome movement. A more global view of the plant meiosis mechanisms is thus emerging, as described in several recent reviews (Mercier and Grelon, 2008; Berchowitz and Copenhaver, 2010; Harrison et al., 2010; Osman et al., 2011). Such a global understanding of these meiotic mechanisms, beyond its intrinsic scientific interest, opens up novel applied possibilities. In this review, we describe how tinkering with the basic mechanisms of meiosis provides potential new technologies to control plant reproduction, such as mastering the level of recombination, manipulation of the ploidy of gametes, re-creation of parental plants from a hybrid, and the implementation of clonal reproduction through seeds.

Wild-type meiosis: how to make recombined haploid gametes

Two major features define meiosis: recombination that produces a patchwork of the pre-meiotic chromosomes and reduction of ploidy by half (Fig. 1). Ploidy is reduced because two rounds of chromosome distribution follow a single replication. Homologous chromosomes are separated at the first division, and sister chromatids are separated at the second division. To be
more accurate, this statement is correct because the chromosome identity is arbitrarily defined by its centromere. Fig. 1 shows that, at the centromere, chromosome distribution follows this rule, but that away from the centromeres the occurrence of crossovers (COs), which are exchanges of continuity between two homologous chromatids, modifies this pattern. At both divisions, entities that will be separated must be physically connected beforehand. In the case of the first division, COs provide this link in partnership with sister chromatid cohesion (ensured by cohesins, a molecular glue that is established at the onset of their replication). This mechanical role of COs is crucial to understand and to tinker with meiosis. This is illustrated in Fig. 1. The two sister chromatids are held together by cohesins. A CO converts this inter-sister cohesion into an inter-homologue link. Hence, if the CO on Fig. 1 is absent, there is no longer a link between the homologous chromosomes (Grelo
c et al., 2001). In such a case, chromatids segregate randomly generating a high level of unviable aneuploid gametes. At anaphase I, sister chromatid cohesion is released from the chromosome arms, freeing the recombinant homologous chromatids to migrate to opposite poles. However, sister chromatid cohesion is kept in centromeric regions and provides the link between the sister chromatids that is required for their segregation at the second division. The centromeric cohesion is released at anaphase II, allowing the distribution of sister chromatids.

Thus, for a correct meiotic division, certain requirements must be met: (i) at least one CO must occur per pair of homologues (the so-called obligate CO) (note that only one chromatid from each homologue is engaged in any given CO, and thus, even with the obligate CO rule, some chromatids can be CO free); (2) cohesion between sister chromatids must be released along the arm at anaphase I but maintained at centromeres until being released at anaphase II; (iii) sister chromatids must co-segregate at the first division and separate at the second division; (iv) the rule of the mitotic cell cycle must be bent to permit two divisions to follow a single replication.

Now, with the knowledge of how wild-type meiosis makes recombinated haploid gametes, let us see how we can tinker with it.

**Can CO formation be controlled?**

We explained above that there is at least one CO per homologue pair. Surprisingly, in most species, the frequency of COs per pair of homologues does not deviate far above 1, typically in the range of 1–4 (chromosome genetic size of 50–200 cM), plants being no exception (see, for example, Giraut et al., 2011; Bowers et al., 2012; Cloutier et al., 2012; Zou et al., 2012; Hudson et al., 2012; Sim et al., 2012; Zhang et al., 2012). This low level of COs occurs despite a large excess of DNA double-strand breaks, which are the precursors of COs [estimated to be 20–40-fold in plants, with for example 230 double-strand breaks (estimated with DMC1 foci) for about 11 COs in Arabidopsis (Anderson and Stack, 2005; Chelysheva et al., 2007; Giraut et al., 2011)]. In addition, COs can also be largely confined to certain regions. In many species, a large proportion of the genome (proximal to the centromere) is CO free, whilst there are many genes within these hyporecombinogenic regions (e.g. in melon and tomato;
Garcia-Mas et al., 2012; Sim et al., 2012; The Tomato Genome Consortium, 2012). Another constraint on CO distribution is interference that prevents two close COs from occurring in a single meiosis. Hence, interference prevents the recovery of double recombinants in a gamete, therefore easily adding one generation to the introgression of a chromosomal fragment of a limited size from one variety to another. Furthermore, recombination tends to be inhibited in crosses involving distant relatives, whilst often desirable traits would ideally be introgressed into elite varieties from exotic germplasm (Gur and Zamir, 2004). Because the CO number and distribution is tightly regulated, this limits the genetic diversity that could otherwise be created in breeding programmes in addition to limiting mapping and positional cloning power in research. Thus, there is a clear interest for breeding programmes to be able to control the many aspects of recombination (Wijnker and de Jong, 2008). A number of recent studies have provided clues how this may be achieved.

**Homologous recombination**

There is evidence showing that some simple methods can influence recombination rates. For example, using a very sensitive assay that measures male recombination in Arabidopsis, it has been shown that CO frequency can be increased slightly by using flowers from secondary and tertiary branches rather than from the primary bolt, or by elevating temperature (Francis et al., 2007). A more pronounced effect is seen when comparing male with female in Arabidopsis: on average, male COs outnumber female COs by 67%, with most of the differences coming from subtelomeric regions. Hence, if you require a recombinant in Arabidopsis, it would probably be best to use a male as the source of gametes, using flowers from the secondary or tertiary branch from plants grown at 28 °C. It will be interesting to test whether these effects are seen in other plant species.

Natural variation has also been identified as a factor that can influence CO frequency in a number of species such as maize, barley, rice, soybean, Arabidopsis, cow, and mouse (Stefaniak et al., 2006; Yandeau-Nelson et al., 2006; Esch et al., 2007; Bovill et al., 2009; Li and Pfeiffer, 2009; Sandor et al., 2012). This suggests that naturally occurring alleles that convey increased recombination frequencies could be stacked into a single genotype to increase or decrease CO rates. A key factor here will be to identify the underlying factors that regulate the frequency of homologous recombination. Recent results also suggest that manipulating karyotype composition could be a way to increase CO frequency in Brassica (Leflon et al., 2010). Indeed, comparing recombination between two homologues in the presence or absence of an extra set of homoeologous chromosomes (i.e. an allotriploid) showed a staggering six times average increase compared with the euploid control. Whilst the mechanism behind this effect remains elusive, it is a tool that can be used in Brassica and potentially other plant species. Changes in ploidy level have also been associated with an increase in recombination rate (~1.5-fold) in Brassica species (Leflon et al., 2010) and in Arabidopsis (Pecinka et al., 2011), where the rates of meiotic recombination were higher in tetraploids compared with diploids. This observation also holds true when comparing the size of genetic maps between diploid and natural allotetraploid Gossypium species (Desai et al., 2006), suggesting that it may be a general trend.

Whilst many genes involved in CO formation have been identified (Osman et al., 2011), no reports that we are aware of have indicated that tinkering with only these elements can lead to an increase in CO frequency. A possible explanation for this may be that known pro-CO genes are not controlling CO frequency, but rather are part of the indispensable machinery that form the COs in wild-type. However, these elusive factors that regulate CO number and distribution are starting to be discovered and can be offered to plant breeders. MSH2 (MutS homologue 2) is part of a highly conserved DNA mismatch repair system that can prevent recombination between DNA sequences that are not perfectly homologous. This can occur meiotically and somatically in species as diverse as yeasts, mosses, and flowering plants (Hunter et al., 1996; Emmanuel et al., 2006; Trouiller et al., 2006). It has been shown in an Atmsh2 mutant that meiotic recombination is increased by ~40% between two polymorphic Arabidopsis accessions, in one interval (Emmanuel et al., 2006). Hence from a plant breeding perspective, the mismatch repair system is probably part of the meiocyte’s arsenal that prevents recombination between distantly related species. Exploiting this knowledge could allow a better control of introgression of foreign germplasm into elite varieties. Another mechanism that could be targeted to influence CO formation is the DNA methylation machinery. Indeed, the DNA hypomethylation Arabidopsis mutant Atmet1 (methyltransferase1) shows elevated centromere-proximal COs, decreased peri-centromeric COs, and increased distal COs (Yelina et al., 2012). Furthermore, Atmet1 and Atddm1 (decreased DNA methylation 1) mutants and Atddm1-derived epigenetic recombinant inbred lines show altered global distribution, whilst the final genome-wide CO frequency is unchanged, thus demonstrating that CO distribution can indeed be modified (Colomé-Tatché, 2012; Melamed-Bessudo and Levy, 2012; Miroouze et al., 2012; Yelina et al., 2012).

Finally, the mutation of a single gene, AtFANCM, has been shown to increase meiotic recombination on eight intervals by a factor of three (Crismani et al., 2012). FANCM is a conserved helicase that also limits meiotic COs in the yeast Saccharomyces pombe (Lorenz et al., 2012) and, given the conservation of function of FANCM between two species as distant as Arabidopsis and S. pombe, it is reasonable to speculate that mutation of FANCM would have the same hyper-recombinogenic effect in other plants species. Interestingly, the extra COs that occur in fancm mutants are non-interfering (i.e. distributed independently from each other) (Crismani et al., 2012), which could facilitate the introgression of small fragments of chromosomes rather than undesirably large blocks. Tripling CO frequency (from around two to around six per chromosome) does not affect meiotic chromosome distribution and fertility of the plant, which leaves the door open to see how high CO frequency can be increased by stacking all the effects known or to be discovered. We have no reason to think that plant species are anywhere near the limit of COs that the chromosomes can tolerate. Most plant chromosomes receive an average of two COs per meiosis (e.g. Giraut et al., 2011; Bowers et al., 2012; Cloutier et al., 2012; Zou et al., 2012).
et al., 2012; Hudson et al., 2012; Sim et al., 2012; Zhang et al., 2012). However, for example, the biggest chromosomes of chicken (200 Mb) (Groenen et al., 2009) or budding yeast (1.5 Mb) receive an unusual average of around ten COs per meiosis. Simply applying the yeast density of COs per Mb, bread wheat chromosome 3B, which measures 1 Gb, would receive more than 6000 COs. It is hard to believe that such an extreme situation could be reached, but it is equally difficult to predict towards what limit the number of COs can actually be manipulated.

**Homoeologous recombination**

Wild or distantly related species present a potential reservoir of genetic diversity that could be introgressed into a species of interest in a breeding programme. However, meiosis is a species barrier that frequently prevents exactly this type of recombination, which is termed homoeologous recombination. Homoeologous sequences are sequences that were once homologous in a common ancestor but have subsequently diverged during speciation, and a hybridization event has brought these sequences back together in the same cell. These homoeologous sequences can align and interact (similar to the way two homologues would) and they can form COs. However, overruling mechanisms typically prevent this homoeologous recombination. Indeed, these mechanisms are required to ensure fertility and to maintain genome stability in allopolyploids where multiple similar genomes are present in the same nucleus. One of the best known examples is the Ph1 locus in allohexaploid bread wheat (Riley and Chapman, 1958; Sears and Okamoto, 1958; Riley et al., 1960; Griffiths et al., 2006), which prevents recombination between homoeologous chromosomes, and hence this polyploid shows diploid-like behaviour at meiosis. The naturally occurring mechanisms that prevent homoeologous recombination provide a major stumbling block for breeding programmes, as not only can introgressing a trait (gene) from wild germplasm be very difficult but it can also be equally so when backcrossing out undesirable linked traits in subsequent generations.

Fortunately, some tools exist to partially mitigate these constraints, e.g. deletion of the Ph1 locus in bread wheat allows some recombination between homoeologous regions, which for many decades has been used in plant breeding programmes. For example, a ph1 mutant was exploited to break the linkage between a desirable and an undesirable trait that were introgressed into bread wheat from Imperial rye (Anugrahwati et al., 2008). The Ph1 locus was reduced to a cluster of CDC2-like genes (Griffiths et al., 2006), which have homology to mammalian CDK2. Subsequently, by exploiting the knowledge that globally the CDC2-like genes are overexpressed in ph1 (Al-Kaff et al., 2008), an appropriate chemical treatment was tested in an attempt to phenocopy the effects of ph1. An okadaic acid treatment, which stimulates the effects of CDK2, obtained promising results on metaphase I spreads, which showed increased chromosome interactions in wheat–rye hybrids (Knight et al., 2010). Another potential tool would be to develop wheat lines containing the dominant Aegilops speltoides genes that suppress Ph1 activity, thereby allowing increased gene transfer (Dvorak et al., 2006). The advantage of developing methods such as the okadaic acid treatment and Aegilops speltoides-derived suppressors is that they are readily reversible when compared with breeding strategies relying on recessive mutations (e.g. ph1 deletion mutants). This is important because, as mentioned above, Ph1-like machinery is essential for maintaining fertility and genome stability in allopolyploids and its function must be restored after the desired recombinants are obtained. Hence, these strategies may provide plant breeders with simpler means to increase introgression of alien chromatin into elite varieties. Indeed, mechanisms that ensure diploid-like behaviour at meiosis and hence fertility are not limited to bread wheat. In Brassica napus, it was discovered that there was natural variation for homoeologous CO frequency, with a major locus called PrBn (Jenczewski et al., 2003; Nicolas et al., 2009; Cifuentes et al., 2010). The locus has not yet been cloned but could be used to tinker with homoeologous recombination.

**Making diploid gametes**

Diploid (2n) gametes are abnormal gametes, as they have the somatic level of ploidy rather than half. If such gametes participate in fertilization, this leads to an increase in ploidy of the offspring compared with the parent. It is generally accepted that whole-genome duplications that occurred in the evolution of many lineages of eukaryotes, and particularly frequently in plants, originated from sexual polyploidizations through 2n gametes (Ramanna and Jacobsen, 2003). From an applied perspective, diploid gametes can allow crosses between related species with different levels of ploidy. This has been used to transfer genetic diversity from diploids, through 2n gametes, to polyploid crop varieties, as demonstrated for example in potato and alfalfa (Peloquin et al., 1999; Ramanna and Jacobsen, 2003). Fertilization involving 2n gametes, could be also used to produce synthetic vigorous tetraploid or triploid individuals, whose sterility is a suited character in some species (e.g. seedless banana, watermelon, and oyster) (Heslop-Harrison and Schwarzacher, 2007). In addition, 2n gametes can be used to produce innovative powerful mapping populations (Van Dun and Dirks, 2006). Various meiotic defects (see below) can lead to the production of diploid gametes. It has been known for a long time that 2n gamete production is under genetic control, and a series of mutants producing 2n gametes has been described in various plants (Ramanna and Jacobsen, 2003; Cai and Xu, 2007), but only a few responsible genes have recently been identified, shedding light on the molecular control of 2n gamete formation.

Making diploid gametes via spindle orientation defects

The first cloned gene whose mutation leads to the production of diploid gametes at a high frequency is Arabidopsis thaliana PARALLEL SPINDLE 1 (AtPS1) (d’Erfurth et al., 2008). The same phenotype was subsequently observed in jason mutants (Erilova et al., 2009; De Storme and Geelen, 2011). In wild-type male meiosis of Arabidopsis, no cytokinesis is observed before telophase II, meaning that the two sets of chromosomes formed after the first division remain in a common cytoplasm. During
metaphase II, the two spindles are roughly perpendicular to each other, leading to four well-separated poles at anaphase II. The AtPS1 and jas mutants present fused or parallel spindles in male meiosis II (Fig. 2A). Thus, the two sets of chromosomes that were separated during the first division are regathered into a single metaphase plate. This creates a situation where it is as if the first segregation did not occur, and the resulting 2n gametes are called first division restitution (FDR) gametes but with recombination. Because the fusion of the spindle is not complete in all meiocytes, ~60% of gametes are diploid but ~40% are haploid. When using genetics to analyse products of meiosis in Atps1 or jas, a mitotic-like division is observed but with meiotic recombination occurring: the recombined sister chromatids are segregated to opposite poles. The resulting 2n gametes are heterozygous at the centromeres (because sister centromeres are separated from one another, with one copy of each homologous chromosome in each gamete) and tend towards random segregation at loci away from the centromeres because of recombination (66% of heterozygosity, i.e. from an A/a parent, the genotype of non-centromeric loci would be two-thirds A/a, one-sixth AA, one-sixth aa) (Fig. 2B). As Atps1 and jas affect only male meiosis, spontaneous triploid progeny is recovered after self-pollination, resulting from the fusion of 2n male gametes with 1n female gametes. Whilst the molecular function of AtPS1 remains elusive, it has been suggested that JASON positively regulates AtPS1 transcription levels (De Storme and Geelen, 2011).

Making diploid gametes by skipping the second division

Another way of producing diploid gametes is simply to skip the second division (giving so-called SDR gametes, for second division restitution). Only two genes whose mutation gives this phenotype have been isolated. The Arabidopsis osd1 (omission of second division) and tam (initially described as tardy asynchronous meiosis; Magnard et al., 2001; Wang et al., 2004) mutants skip the second meiotic division, producing dyads instead of tetrads of spores (d’Erfurth et al., 2009, 2010; Wang et al., 2010). In both osd1 and tam mutants, prophase and meiosis I are indistinguishable from the equivalent stages in the wild type with recombination and segregation of homologous chromosomes. From there, the resulting two sets of chromosomes will constitute the genome of the gametes, omitting the second meiotic division (Fig 2A). As these mutations affect both male and female meiosis, 4n plants are recovered when selfed. Both genes are components of the cell-cycle machinery, and contribute to the cell-cycle-specific regulation that allows a second division to occur without an intervening replication at meiosis. TAM is one of the ten A-type cyclins of Arabidopsis thaliana (CYCA1;2). TAM is involved in all the meiotic cell-cycle transitions (Bulankova et al., 2010; d’Erfurth et al., 2010; Cromer et al., 2012). OSD1 is a regulator of the anaphase promoting complex (Cromer et al., 2012; Iwata et al., 2011), a conserved component of the cell cycle machinery, which notably destroys cyclins to promote exit from mitosis or meiosis (Cooper and Strich, 2011). Genetically, as the sister kinetochores migrate together at meiosis I in tam and osd1, the diploid gametes are systematically homozygous close to the centromeres (Fig. 2B).

Away from the centromere, recombination distributes the alleles towards randomness (66% of heterozygosity).

Making diploid gametes by turning meiosis into mitosis

Finally, diploid gametes can be produced by turning meiosis into a single, mitotic-like division. As such, any heterozygosity present in the parent is transmitted into the 2n gamete. This is observed in mutants of several genes. In maize, the dominant mutation of AGO104 (which belongs to the ARGONAUTE multigene family acting on transcriptional and post-transcriptional gene silencing as well as on RNA interference) leads to the production of 30–40% of unreduced gametes in both males and females (Singh et al., 2011). A histone modification marker, used to discriminate meiosis from mitosis, suggests that meiocytes in ago104 undergo mitotic-like division rather than a meiotic division. Interestingly, maize AGO104 is an orthologue of AGO9 of Arabidopsis, and Atago9 mutants show spontaneous development of somatic cells into functional diploid gametes (completely skipping meiotic development, so-called apospory) (Olmedo-Monfil et al., 2010). Other maize mutations in the epigenetic machinery can induce phenotypes reminiscent of mitotic-like divisions at meiosis (Garcia-Aguilar et al., 2010).

The SW11/DYAD/DSY10 gene was identified more than a decade ago, and its molecular function remains elusive, despite the characterization of a range of mutant alleles (Siddiqi et al., 2000; Mercier et al., 2001, 2003; Xue and Makaroff, 2001; Agashe et al., 2002; Boateng et al., 2008). Whilst male meiosis is variously affected, female meiosis appears to be similarly modified in all of them. Indeed, in female meiosis, ten univalents are observed, with a balanced segregation of the sister chromatids, mimicking mitosis. The fertility of these plants is massively reduced, but among the few seeds produced, 78% originate from a mitotically derived female gamete (Fig. 2A) (Ravi et al., 2008). The same phenotype is observed in maize when AM1, the orthologue of SW11/DYAD is mutated (Pawlowski et al., 2009). Furthermore, it has been shown that the rare spontaneous descendants of dyad are triploid, resulting from the fertilization of a mitotically derived unreduced female gametophyte by reduced pollen (Ravi et al., 2008).

By combining mutations that abolish each of the features that distinguish meiosis from mitosis, 2n mitotic-like gametes can be obtained at a frequency of 100% (d’Erfurth et al., 2009). The first feature is recombination, which can be eliminated by mutating the very conserved function of SPO11 (Grelon et al., 2001). SPO11 is essential for the formation of DNA double-strand breaks and thus the initiation of meiotic recombination. Hence, in a spo11 mutant, no recombination can take place, leading to the random segregation of ten univalents. Secondly, to convert meiosis into mitosis, sister kinetochores (and hence sister chromatids) must have a bipolar orientation rather than a monopolar orientation as they would during meiosis. Mutation of RE8 can satisfy this need. REC8 is a protein that ensures sister chromatid cohesion in meiosis (Bai et al., 1999; Bhatt et al., 1999). Hence, a spo11 rec8 double mutant shows a balanced segregation of the two sets of ten sister chromatids in meiosis I (Chelysheva et al., 2005), mimicking a mitosis at first division. However, as the second division occurs in this double mutant, the resulting...
Fig. 2. Mutants producing diploid gametes in *Arabidopsis thaliana*. (A) Schematic representation of chromosome behaviour at mitosis and at meiosis in the wild type and in mutants producing diploid gametes. For simplification, only two pairs of chromosomes are represented. (B) Genetic content of the resulting diploid cells or gametes. The frequency of heterozygosity in the gametes (i.e. from an A/a parent, % of diploid gametes being A/a, and neither A/A nor a/a), is represented according to the position of the genetic marker along the chromosome. During mitosis in diploid cells, chromosomes replicate and sister chromatids segregate to opposite poles and produce two diploid daughter cells. The resulting cells are identical to the mother cell, retaining 100% heterozygosity from the mother cell all along the genome. During meiosis, replicated homologous chromosomes pair and engage in recombination and then segregate to opposite poles. Sister chromatids are separated at meiosis II, and four recombined haploid spores are formed. During meiosis in *Atps1* or *jas* mutants, recombined chromosomes are segregated normally during the first division but are pooled back together on a single metaphase II plate because of parallel or fused spindles. Sister chromatids are then segregated during anaphase II. This mechanism mimics the absence of first division, as only the segregation of sister chromatids is effective, but recombination has occurred. Consequently, heterozygosity is conserved close to the centromere (all the gametes are A/a at the centromeric loci) but is decreased towards the telomeres (two-thirds Aa and one-third A/A or aa). In contrast, in *osd1* or *tam* mutants, the first division occurs normally and the homologous chromosomes are separated, but omission of the second meiotic division enables sister chromatids to remain together in the same daughter cell. Thus, the heterozygosity of the parent is lost at the centromeres (all the gametes are either A/A or a/a, but never A/a at the centromeric loci). However, COs shuffle the chromatids and the frequency of heterozygote gametes (A/a) tends towards two-thirds at loci away from the centromeres. Finally, in a *dyad* or *MiMe* mutant, meiosis is replaced by a mitosis-like division (with a higher efficiency in *MiMe*). The genetic information (notably heterozygosity at all loci) from the mother cell is conserved all along the chromosomes, as neither recombination nor the second meiotic division has occurred.
free chromatids are unable to align at metaphase II and segregate randomly. Thus, the last feature that has to be removed to convert meiosis into mitosis is the occurrence of a second division, which can be achieved by mutating OSD1 or TAM (see above). In a triple spo11 rec8 osd1 (d’Erfurth et al., 2009) or spo11 rec8 tam (d’Erfurth et al., 2010) mutant, the two sets of ten chromatids segregate perfectly once, and as no further division occurs, diploid gametes are obtained via a mitosis-like division instead of meiosis (see Fig. 1 in d’Erfurth et al., 2009, for a graphical explanation). Hence, this triple mutant, spo11 rec8 osd1, is called MiMe (mitosis instead of meiosis), and performs mitosis instead of meiosis at a rate of 100%. Self-fertilization involving the clonal 2n gametes leads to doubling of ploidy at each generation.

Genetic content of unreduced gametes differs

According to the mechanism of 2n gamete production, their genetic content may differ dramatically (Fig. 2B). This must be taken into account from an applied perspective to maximize the transfer of desirable alleles. There are two main features controlling the genotype of diplogametes: segregation of homologous or sister chromatids (more accurately their centromeres) and the presence or absence of recombination. In the case of a mitotic-like division (e.g. dyad, MiMe), sister chromatids segregate without recombination, making 2n gametes genetically identical to their parent. In the case of Atps1 and jas, sister centromeres also segregate from each other, but in this case recombination occurred. Thus, heterozygosity is conserved at centromeres (if the parental plant was A/a, 2n gametes would be systemically A/a) and tends to be reduced towards randomness (two-thirds A/a, one-sixth AA, one-sixth aa) away from the centromeres (Fig. 2B). The opposite effect is seen in osd1 or tam mutants, where sister centromeres migrate together and recombination occurs. Thus, heterozygosity is completely lost at the centromeres (i.e. as the diploid parental plant is A/a, 2n gametes will be either A/A or a/a but never A/a). Away from the centromeres, and because of recombination, the genetic composition of unreduced osd1 and tam gametes tends towards randomness (two-thirds heterozygous).

Getting rid of recombination: how to fix the ideal genotype

Hybrids often have better field quality than their parents. This phenomenon, called hybrid vigour or heterosis, is widely exploited in plant and animal breeding, although its mechanism remains elusive (Chen, 2010). F1 hybrids must be recreated continually because genetic segregation eliminates half of the heterozygosity in the F2 (97% by the F6) and would thus rapidly dilute the heterosis effect.

Reverse breeding

In traditional plant breeding, to take advantage of heterosis, the selection is applied to two homozygous lines on the ability to produce the best hybrid (F1) when crossed. The plants that are selected and the ones that express the sought phenotype are thus not the same. Reverse breeding is a novel plant-breeding strategy that proposes a top-down approach. First, the best hybrid is selected and the parental lines are then recreated (Dirks et al., 2009). This strategy produces homozygous parental lines from any heterozygous plant and is based on the abolition of CO formation in the heterozygote and the production of doubled haploid plants from the gametes free of COs. Selecting and crossing two lines with complementary sets of chromosomes allow the production of the hybrid on a commercial scale. This technique also allows the production of so-called substitution lines that are, for example, heterozygous for only one chromosome, facilitating the selection of the best alleles carried by this chromosome. The feasibility has been demonstrated recently in the model Arabidopsis (Wijker et al., 2012), using the extinction of DMC1, a gene required for CO formation in most eukaryotes, to abolish CO formation. Due to the loss of most, if not all COs, chromosomes segregate randomly, but balanced gametes are produced at a rate close to the expected rate (0.5^x, where x=chromosome number). These gametes have been turned into haploid plants using centromere-mediated genome elimination (Ravi and Chan, 2010) and then self-pollination resulting in double-haploid diploid plants that are homozygous at all loci in the genome. From these, both the original hybrid and a set of substitution lines have been obtained. The technique is now therefore a reality; however, it can only be applied to crops with no more than 12 chromosomes, due to the difficulty in obtaining balanced gametes by chance and in which spores can be regenerated into doubled haploids.

Apomixis

Apomixis is the asexual formation of a seed from maternal material. Importantly, this means that meiosis is replaced by mitosis and theoretically that heterozygosity could be maintained through generations. This would provide a major simplification of the production of hybrids that could be propagated indefinitely without losing the benefits of heterosis (Bicknel and Koltunow, 2004).

Many plant species reproduce via apomixis (>400 known species). However, there is an under-representation of important crop species that reproduce that way. This is speculated to have occurred by chance through human selection early in the history of agriculture (Bicknel and Koltunow, 2004). Therefore, the identification of the underlying genetic mechanisms represents a holy grail for a simple way of increasing crop yields, and global efforts are progressing towards identifying the genes required for apomixis. It has been established that apomixis is under the genetic control of a limited number of loci, but the corresponding genes have not yet been identified (Pupilli and Barcaccia, 2012; Koltunow et al., 2011a,b). So far, attempts to directly introgress apomixis into crops have been unsuccessful, making the identification and understanding of natural apomixis mechanisms crucial.

Another opportunity to introduce apomixis into plants exists through the possibility of engineering apomixis de novo by tinkering with sexual processes. The first step is to modify meiosis by turning it into a mitotic-like division, producing clonal
gametes with the somatic level of ploidy as described above in Arabidopsis (dyad, MiMe, ago104, ago9). The second step is to produce seeds from these unreduced gametes, without any genetic contribution of another gamete. This could be achieved through parthenogenesis, or by post-fertilization elimination of the unwanted chromosomes. The Arabidopsis GEM (genome elimination) line provides the latter possibility, as the genome of the GEM line is eliminated post-fertilization when crossed with any other genotype (Ravi and Chan, 2010). Indeed, when GEM was crossed with dyad or MiMe, clonal seeds were recovered (Marimuthu et al., 2011). This established that it is possible to mimic apomixis by tinkering with sexual processes. However, the current system has some limitations, because it is still cross dependent and does not recover clones at 100%. Future challenges include increasing the efficiency of clone production, reducing the dependence on a cross, and making the system inducible.

Conclusion: the complete design

From the naïve view of a molecular geneticist, breeding can be summarized in two steps: to mix and to fix. The complete design would thus include a step of intense mixing (increased recombination) to provide new elite genotypes that combine as many traits as desired, and then a step to fix and propagate the supreme individual at an industrial scale (apomixis or reverse breeding). Some genetic strategies have emerged recently to address these two issues, and one of the next challenges is to combine them. The other challenge is to transfer these technologies from model organisms like Arabidopsis to species as complex as wheat or organisms like Triticum aestivum.

Some obstacles stand in the way: the number of copies of each gene to tinker with due to polyploidy and the appropriate tools such as sequence information and mutant resources, along with generation time and space limitations. However, there is no reason why the novel and future concepts developed in model plants could not be applied to crops.

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